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Adoptive Cell Therapy and chimeric antigen receptor – T cells

Adoptivna celična terapija in limfociti T z izraženimi himernimi antigenskimi receptorji

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Abstract

In cancer therapy, currently used approaches include surgery, radio- and chemotherapy and various combinations of these. Immunotherapy is emerging as the 'fourth pillar' of cancer therapy. Science Magazine declared cancer immunotherapy as a 'breakthrough of the year' in 2013. While antibody therapies and cytokines have been in use for a longer period of time, the cell immunotherapies are only gaining momentum in recent years, especially thanks to adoptive T-cell therapy (ACT). Adoptive T-cell therapy, which is subject of this article can be divided mostly to therapies with tumor-infiltrating lymphocytes (TIL) and to therapies using T-cells genetically modified with transgenic T-cell receptors (TCR) or chimeric antigen receptors (CAR). All of these therapies are exclusively personalized and are at the moment based on the autologous transplant of the patient's own cells. Therapeutic cells are prepared for each patient individually, which carries a great impact on the processes of their development and production, as well as the logistics and costsinvolved. The new mode of therapies with CAR-T, for specific diseases at the moment the only registered mode of ACT, causes revolutionary breakthroughs in the development of such advanced therapeutics and, consequently in clinical oncology. CAR-T therapy is achieving an incredible success in clinical practice especially in combat against hematological cancers, while the treatment of patients with solid tumours it has not been as successful. Along with that, due to great complexity of the CAR-T therapy, it is accompanied by frequent and severe side effects which can be fatal in worst cases. By adopting adequate measures, these effects can be controlled and partially mitigated. CAR-T therapy is being introduced to Slovenia through registered, commercially accessible therapeutics of this kind, while the access to other ACT is still pending. At the same time, in Slovenia in this field, we are developing our own knowledge and technology, hoping that new, efficient treatment modalities become accessible to a wider population of patients as soon as possible.

Izvleček

Pri zdravljenju raka se trenutno uporabljajo pristopi kirurškega zdravljenja, radio- in kemoterapije ter različne kombinacije teh pristopov. Kot 'četrti steber' onkološkega zdravljenja se razvija imunoterapija. Revija Science je imunoterapijo raka že leta 2013 razglasila za 'preboj leta'. Terapije s protitelesi in citokini se uporabljajo že dlje, celična imunoterapija pa pridobiva na pomenu šele v zadnjih letih, še posebej po zaslugi adoptivne T-celične terapije (ACT). Terapije z adoptivnimi limfociti T, kar obravnava prispevek, delimo predvsem na terapije s tumor infiltrirajočimi limfociti (TIL) in terapije z limfociti T, ki so gensko spremenjeni bodisi s transgenimi T-limfocitnimi receptorji (*angl.* T-cell receptor, TCR) ali pa s himernimi antigenskimi receptorji (CAR). Vse te terapije so izrazito personalizirane in trenutno temeljijo na presaditvi avtologni, t.j. bolnikovih lastnih celic. Terapevtiki se pripravijo za vsakega bolnika posebej, kar zelo vpliva na procese razvoja in izdelave ter tako na logistiko in stroške, ki so povezani s tem. Nov način terapij s CAR-T, ki

je za določene bolezni trenutno tudi edini registrirani način ACT, povzroča revolucionarne premike pri razvoju takih naprednih terapevtikov, s tem pa tudi v klinični onkologiji. Terapija s CAR-T dosega izredne uspehe v klinični praksi zlasti v boju proti hematološkim oblikam raka, medtem ko pri zdravljenju bolnikov s solidnimi tumorji še ne. Hkrati pa zaradi velike kompleksnosti terapije težavo pri zdravljenju s CAR-T povzročajo pogosti, težki stranski učinki, ki lahko vodijo tudi v bolnikovo smrt. Z ustreznimi ukrepi lahko stranske učinke nadzorujemo in delno ublažimo. Terapija s CAR-T vstopa v Slovenijo s komercialno dostopnimi terapevtiki te vrste, na možnost ostalih adoptivnih celičnih terapij pa bo potrebno še počakati. V Sloveniji pa na tem področju razvijamo tudi lastno znanje in tehnologijo, zato upamo, da bodo novi učinkoviti načini zdravljenja čim prej dostopni čim širšemu krogu bolnikov.

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1 Introduction

In cancer, the patient's immune system limits the oncogenesis and tumour progression, but it also plays an important role in the response of existing tumours to anti-tumour therapy, which is reviewed in (1). With the objective of inciting or renewing immune system's capabilities to remove malignant T cells in a specific way, numerous forms of anti-tumour immunotherapy have been developed (2-4). This means that activation of one's own immune system to recognise tumour cells and destroy them is one of the key objectives of cancer immunotherapy. In literature, cancer immunotherapy is most frequently – however not exclusively – connected with (5): antibody therapy (6) and immunostimulatory cytokines (1), anti-tumour vaccinations (1,7-9), and with adoptive cell therapy (1,5). Adoptive cell therapy has been gaining traction in the past few years, especially due to therapy with T-cells, which had been genetically modified with chimeric antigen receptors (CAR-T). CAR-genetically-modified T cells use synthetic biology to combine the functionalities of the humoral and the cellular immune response, which in such combination does not exist in nature. This method of therapy is causing revolutionary shifts in the development of drugs, and consequently in clinical oncology. The first such drugs were registered in 2017 with the US Food and Drug Administration (FDA), and in 2018 with the European Medicines Agency (EMA), and are therefore already available in Europe.

The state of clinical studies shows that the range of such medications on the market will increase quickly over the next few years. CAR-T therapy has been achieving exceptional success in clinical practice, especially with haematologic types of cancer (10). The situation is significantly worse in treating patients with solid tumours (11), also because of the micro-environment that does not favour CAR-T cells. An additional issue with CAR-T therapy are the frequent severe side effects, which can even result in the patient's death, and that applies to both haematologic types of cancer, and to solid tumours.

2 Adoptive T-cell therapies

Main therapeutic approaches in cancer cellular immunotherapy include approaches under the common name adop-

tive T-cell therapy (ACT), sometimes also named adoptive cell transfer (ACT). This is immunotherapy based on naturally present or genetically modified cells, which can achieve regression of cell tumours even in metastatic cancer (12).

With these approaches, specific T cells are isolated, then reproduced and injected into the patient (13), to identify and target tumour cells and destroy them. ACT has numerous advantages that make it useful for treating cancer: 1. T-cell response is specific and can even differentiate between a healthy cell and a tumour, 2. After specific activation, T cells can reproduce by clonal expansion, 3. T cells can travel to the site of the antigen, 4. Memory T cells can maintain the therapeutic effect long after the start of therapy (14). The key breakthrough in the development of ACT technologies is laboratory identification of specific tumour associated antigens (TAA) that allow a patient's immune cells to differentiate between cancerous and healthy cells (15) by not causing any damage to the healthy tissue, namely by: 1. Identifying antigens which are excessively expressed in tumour cells, when compared to the healthy ones, 2. Identifying the neoantigens which are expressed in tumour cells as the result of somatic mutations, and 3. Identifying antigens on tumour stem cells (14). TAA identification and new findings in clinical immunology of cancer patients have made it possible to develop numerous ACT modes in which the autologous or allogenic (1) T cells are harvested from the patient's blood or tumour in various procedures and stimulated to reproduce in *in vitro* conditions. Reproduced this way, they are injected back into the host/patient after lymphodepletion to contribute to the destruction of tumour cells. This procedure is applicable to most patients who do not have an effective immune response against the tumour (1,14). ACT can also be used in combination with chemotherapy and/or other types of immunotherapy in order to improve the reproduction, survival and functionality of infused therapeutic cells, and ensure their evolution in an immunologically permissive environment (1).

Along with the above-mentioned CAR-T therapies, other adoptive T cell therapies include tumour-infiltrating lymphocytes (TIL) treatment, which is reviewed in (16), and therapies with transgenic T cell receptor (TCR)-modified T cells, which are also reviewed in (16). All the above therapies have triggered tumour regression in clinical studies; however, they currently only achieve permanent results in a portion of the patients. Obstacles on different levels are for now limiting the success rate of adoptive T-cell therapies in solid tumours. Advancements in basic molecular biology have made it possible to develop numerous approaches in genetic engineering of T cells at the level of the genome, at the level of RNA and at the epigenetic and protein levels with the objective of pharmacologically improving the immune system (13,17).

The current level of knowledge and technology in combination with different approaches to cellular engineering and modern insights into the biology of T cells and tumour immunity have come to a point where rational engineering of an effective T-cell anti-tumour immunity has become practically feasible and can be clinically tested (13). Below we present the three methods for treatment with T cells.

3 Tumour-infiltrating lymphocytes (TIL)

Tumour-infiltrating lymphocytes (TIL) are the patient's own lymphocytes that have left the blood stream and migrated towards the tumour. Their number depends on tumour type and stage and is sometimes connected with the prediction of the outcome of the disease. TIL can be found in the tumour or stroma, and their tasks can be changed during advancing tumour preogression or as a response to anti-tumour therapy. The presence of lymphocytes in the tumour is often related to

a better outcome prediction in therapy (18-22).

TIL technology has brought the most clinical success in clinical trials in treating solid tumours (23), especially with metastatic melanoma. The results of the first clinical study with TIL on metastatic melanoma was conducted already in 1994 (24). TIL was obtained from approximately 3-centimetre long pieces of metastatic melanoma tissues. After tissue sample resection, TIL was reproduced in tissue cultures with the presence of interleukin-2 (IL2). When enough TIL was obtained, it was tested for identification of autologous melanoma cells; if these were not available, then with a series of HLA-matching melanoma cell lines. The lymphocyte response was tested by establishing the presence of the interferon-γ (IFNγ), secreted into the growth medium using an ELISA test. Only the cultures with TIL that were reacting to the melanoma were selected. The cells were reproduced through stimulation with a soluble antibody against CD3 in the presence of high concentrations of IL2 (6000 IU/ml) and irradiated allogenic or autologous nutrient cells (24).

In the past years we have obtained a better insight into the mechanisms of how TIL operate, especially in relation to the role of the lymphodepletion of T cells in the hosts themselves (patient, therapy recipient), the role of IL2 as a survival factor for injected TIL, as well as the optimum quality of the injected cells and the pattern on how it identifies the antigen (25,26).

Types of cancer from which it was possible to expand the reactive TIL include the tumours of the breast, digestive tract, head and neck, kidneys, lungs, ovaries, and cutaneous and uveal melanoma, which is summed up in (23,25,26). Therapy with reactive TIL after lympho-depleting chemotherapy has caused an objective decrease in tumour across all the above-listed types of cancer, even with patients resistant to therapy with immune-checkpoint inhibitors (23). Even though approximately 50% of melanoma patients respond to therapy with TIL (23,27), the regression of epithelial tumours is present on average with fewer than 15% of patients (23).

4 Transgene T-cell receptors

Natural T-cell receptor (TCR) is a complex, somatically rearranged (23) molecule, acting as a receptor on the surface of T cells, responsible for identifying antigens that are expressed with the major histocompatibility complex (MHC) as peptides. As a heterodimer, TCR consists of two different, highly variable protein chains, most frequently α and β, expressed as part of the complex with a constant CD3 protein (28). TCR cannot transfer the signal independently. When TCR binds to the antigen/MHC complex, the T cell is activated in a complex cascade of signal transduction, which also includes co-receptors, enzymes, and other molecules with which TCR forms non-covalent bonds (23). The TCR binds to the antigen has a relatively low affinity and is degenerated. This means that numerous TCRs can identify the same antigen peptide or that numerous antigen peptides can be identified by the same TCR (29).

Early clinical studies with genetically modified T cells were based on the expression of recombinant, cloned TCRs with a targeted affinity for tumour antigens (30). The first melanoma regression following a therapy with autologous T cells, modified with transgenic TCRs against the MART-1 antigen, was described in 2006 (31). TCRs can recognize both intracellular and extracellular antigens presented on MHC (30). This means it has access to a significantly bigger range of antigens, such as CAR-Ts, which is one of the most important advantages of TCRs over CAR-Ts.

Introducing transgenic TCRs using γ-retro- or lentiviral vectors results in insertion at a random location in the genome of the T-cell. However, the endogenous αβTCR remains flawless, which leads to the expression of various combinations of endo- and exogeneous TCR

heterodimers in the genetically modified cell. Mixed heterodimers of the endo- and exogeneous TCRs can decrease the operation of those with correct dimerizations and cause toxic side effects that can lead to a disease of the implant against the host. A detailed knowledge of the molecular architecture of the natural TCR allows us to make rational modifications to its structure, which improves safety, efficacy, and scalability of TCR-based immunotherapies. In order to improve the safety and/or increase functionality, the structure of transgenic TCRs can be modified in several way. These modifications are: 1. Replacing all or select parts of human sequences with mouse – murinization (32,33), which can lead to immunisation, 2. Cysteine modifications, which enable the second disulphide bond (34), 3. Modifications of hydrophobic properties of the transmembrane region of TCR α (34), 4. Inversion of human TCR α sequences (35), 5. Mutagenesis of complementary determining regions (CDR) for increasing affinity, 6. Strengthening normal TCR heterodimers (23), and 7. Modifying/adapting T-cells using CRISPR/Cas9 technology (36).

By targeted integration of the transgenic TCR to "safe places" in the genome, including the locus for the endogenous TCR, we can bypass numerous safety and functional inhibitions, such as: 1. The integration of the transgene to defined safe places excludes the possibility of issues in the operation of normal genes or genotoxicity, 2. The deactivation of the operation of endogenous TCR disables incorrect TCR dimerization with the above-mentioned consequences, 3. Integration at the location of the endogenous TCR places the transgene under physiological transcription control, which lowers the possibility of tonic signalization and immunologic exertion, and 4. Targeted integration of the transgene using non-viral methods lowers production costs, because making GMP nucleic acids is cheaper than GMP viral particles (23,36).

Researchers have reported success when treating patients with WT-1, positive AML and MDS, with WT-1 specific TCR-T (37).

5 Genetically engineered chimeric antigen receptor T cells – CAR-Ts

T cells can also be reprogrammed, i.e., genetically engineered to express chimeric antigen receptors (CARs) which bind T cells with a specific antigen on the surface of a cancerous or other type of diseased cell. CARs consist of antigen-recognition domain, e.g., a single-chain variable fragment (scFv), a hinge region, the transmembrane domain, and the intracellular T-cell signalling and co-signalling domains, needed for activating T-cells (38,39). With scFv binding to a specific antigen, an intracellular signalling cascade is triggered, activating the T cell against the target tumour cell through the CD3ζ T-cell receptor complex, and one or more co-stimulatory receptors in the intracellular part of CARs (39,40). Introducing a co-stimulatory receptor in the intra-cellular part of second-generation CARs (first generation means CARs without a co-stimulatory receptor) supports a large number of variations with regard to antigen specificity, co-stimulatory signalling domains and components for T cell activation. There are reports of more than 100 specificities for CARs with scFv technology and at least eight different co-stimulatory components (41).

Until now, the most researched are second generation CAR-Ts that include either CD28 or 4–1BB (41). Currently registered (2017 and 2018) CAR-T drugs are based on them.

Until now T cells were genetically engineered with CARs, which can identify different tumour-associated antigens (TAA) independently from HLA, including the B-lymphocyte antigen CD19 marker (38), unaltered proteins, glycoproteins (42), glycolipids (42) and carbohydrates (44).

CAR-T therapy has been achieving exceptional success in clinical practice, especially with haematologic types of cancer (10), and with patients with recurrence, resistant to chemotherapy (45). The first CAR-T-based drugs targeted on CD19+ B-lymphocytes were registered with FDA (2017) and EMA (2018), and are already available in Europe, currently for treating acute lymphoblastic leukaemia with younger patients and for treating some types of non-Hodgkin's lymphoma. The situation is significantly worse in clinical studies testing CAR-Ts for treatment of patients with solid tumours (11), also because of the micro-environment that does not favour CAR-T cells. The most frequent side effects that can lead to a patient's death include the following: cytokine release syndrome (CRS), neurotoxicity (46), tumour lysis syndrome (47). CD19 CAR-T therapy must be conducted according to a special regime of preparing a patient (preconditioning) with the depletion of endogenous lymphocytes, without which we cannot expect appropriate results of the therapy. The toxicity of CAR-T therapy is usually successfully controlled using anti-IL-6 antibodies (one of the main factors for cytokine release syndrome) and corticosteroids, and partially mitigates with the intensity of preconditioning and T-cell doses (41). The results of clinical studies with newer constructs that include low-affinity antigen binding domain against CD19 point to improved proliferation and persistence of such CAR-T cells in paediatric patients and a lower toxicity (48).

The data from more recent clinical studies also show that neurotoxicity of CAR-T therapy can be reduced using completely human CAR constructs (49).

Similarly to the CD19 antigen, clinical studies with CAR-T have also been successful in some other haemato-oncological targets, e.g. CD22 with acute lymphoblastic leukaemia (ALL), and the B-cell maturation antigen (BCMA) in a disseminated plasmacytoma (41,50,51).

These antigens are not only expressed in malignant cells, but also in normal ones, but only in those not of vital importance, i.e., those temporarily expendable (41). This does not apply to other frequently mentioned targets for CAR-T, e.g., mesothelin, transmembrane receptor tyrosine kinase protein (ROR1) or the prostate serum membrane antigen, which are expressed in the part of the normal cellular population. Even lower expression of these antigens in normal tissue can cause statistically significant toxicity (41). An additional issue with solid tumours is their immunosuppressive microenvironment, which is an obstacle for all types of immunotherapy (3). The inter-cellular environment is less favourable to T-cell activity because of hypoxia, necroses, acidoses, lack of nutrients, and a range of immunosuppressive molecules (PD- L1, IL-10, TGFβ, indoleamine-2, 3-dioxygenase). The regime for preconditioning a patient before T-cell therapy reduces the impact of these factors (52). This is usually not enough to remove all inhibiting factors.

At the Department for Research and Development of the Blood Transfusion Centre of Slovenia and in cooperation with the Department of Synthetic Biology and Immunology of the National Institute of Chemistry, and the Haematology department of the University Medical Centre Ljubljana, we have been developing our own approaches to second and third generation CAR-T treatments. These approaches include the development of molecular constructs that can be expressed in T cells with the help of transposon or viral vectors and in RNA form. T cells armed like this are then reproduced in an environment of good laboratory practice, and then their functionalities are verified *in vitro* and *in vivo*.

6 Discussion and conclusions

Cancer immunotherapy is an emerging 'fourth pillar' of cancer treatment. It has been proven that with different types of solid tumours (53), current immunother-

apies do not benefit most patients. This is especially true for patients with moderately mutated tumours arising from epithelial organs (54), which form the leading reason for death from cancer (55). In order for immunotherapy to result in tumour regression, three critical requirements must be met (reviewed in (23)): 1. The patients must have a range of T cells, capable of recognising antigens on the surface of tumour cells. 2. The same antigen-specific T cells must have the ability to reproduce and infiltrate the tumour mass and remain there. 3. T cells must remain functional in the tumour environment. Patients with regular epithelial tumours are faced with numerous significant obstacles that limit the capability of T-cell lines to meet these requirements. Each of the above critical requirements can basically be fulfilled by using adoptive cell transfer (23).

Cellular immunotherapy with adoptive cellular transfer has gained importance in the past years. This is mainly due to the clinical success of the first genetic immunotherapy drugs based on CAR-T for treating ALL and some types of non-Hodgkin's lymphoma.

With their FDA (2017) and EMA (2018) registration, such drugs finally became more accessible to a somewhat broader range of patients. By using CAR-T therapy we can achieve permanent remission with patients suffering from refractory B-lymphoid cancers, while the results of treating solid tumours with CAR-Ts have yielded poorer results (23). Additional strategies of refocusing T-cell specificity and cytolytic activity can improve adoptive cellular therapies and their role in cancer therapy. Even though recombinant antigen TCR and CAR receptors are similar in a certain sense, these two classes of antigen receptors differ by structure, affinity, immune synapse organisation, and the density of target antigens, needed to trigger T-cell functions (23). TCRs are expressed on all T cells and are complementary to CARs; however, sometimes they also have an advantage over them. Unlike CARs, which identify antigens on the surface of cells, independently of MHC (HLA), which represent a minor share of potential protein antigens (56,57), TCRs can identify antigens of any cellular part expressed on MHC, including the membrane, cytoplasm or nucleus. This allows TCRs to identify a significantly broader spectrum of targets, such as neoantigens, antigens of tumour stem cells and viral proteins (23,30). TCRs also respond, as they have the ability to effectively identify and amplify antigen signals onto somewhat lower densities of epitopes than CAR, which are needed for signalising synthetic CAR (23). So far, all transgene TCR- and TIL-based therapies are still in clinical trial phases. The key event in the development of ACT technologies that significantly defines their safety and effectiveness is identifying specific tumour associated antigens (TAA).

Based on these, patient's immune cells can reliably and safely differentiate between cancerous cells and healthy ones (15) without the so-called on-tumour, off-target effect. To put it differently: any potential adverse effects on healthy cells resulting from therapy are not too big of a risk for the patient. The best-known example is anti-CD19 CAR-T therapy, which, along with malignant ones also removes healthy B lymphocytes and lymphoblasts, and causes lymphocyte B aplasia. This can be partially successfully managed with immunoglobulin therapy.

Because ACT is a revolutionary therapy that changes the methods of treating resistant types of cancer, it is also clear that this is a new form of therapy that has been used to treat a fairly small number of patients (using registered drugs). The condition of most patients, participating in clinical studies, has been followed up for a relatively short time. Even so, the amount of data on early responses to the therapy has been growing quickly. The duration of these responses will only become predictable once the state of the patients participating in the studies will be followed up

for a longer time. It is also important that the number of patients participating in clinical studies grows considerably. Only with a larger number of participants, followed up over a longer period of time, will we make new discoveries on the effects of these types of therapies, and how to reduce toxicity, and how to improve the mitigation of side effects.

The current status of clinical studies with ACT shows that the number of ACT-based drugs, or rather, their production, will only increase.

This also means a better accessibility to ACT for patients with the most difficult disease. However, new forms of TCR and TCR-like structures that identify antigen are also in development (23). With such approaches personalised production, ACT and genetic engineering will not be necessary, which will make them more readily available and broaden the use of the therapy.

With the first CAR-T-based drugs, T-cell immunotherapy also makes its way to Slovenia. Even though this is good news, these and similar personalised therapies are related with exceptionally high production costs and along with that, also high costs of treatment and delays that occur from apheresis or tissue harvesting and until the personalised drug is produced and the therapy itself begins. It is a question of whether and when such therapies will be available to the largest possible number of patients.

For several years now, such adoptive cellular therapies are being developed in the translational chain formed by institutions such as the National Institute of Chemistry, Department of Synthetic Biology and Immunology, Blood Transfusion Centre of Slovenia and its Department for Research and Development, and the Ljubljana University Medical Centre's Haematology department.

Translational team brings together world-class synthetic biologists from the National Institute of Chemistry, translation and production capacities, apheresis centre of the Blood Transfusion Centre, and world-class haematologists. With increasing recognition of local know-how and its increasing use, it would be paramount to provide better access to modern therapies, such as adoptive T-cell therapies, to patients in Slovenia and beyond.

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